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# COGNITIVE EFFECTS OF HYPERCAPNIA ON IMMERSED WORKING DIVERS

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Cognitive effects of inspired doses of $CO_2$ during submerged working dives have previously not been explored. Three experiments using male volunteer Navy divers in the NEDU test pool under 12 feet of fresh water explored: (1) dose-related and on/off effects of 1.5% (Phase 1a, N=20) and 3% (Phase 1b, N=16) inspired $CO_2$; (2) questions of whether switching to gas free of $CO_2$ results in further changes in performance or restoration to baseline (Phase 2, N=34); and (3) differences in the effects of $CO_2$ in air vice in $O_2$ (Phase 3, N=16). End tidal $CO_2$ was collected from all divers and correlated with cognitive performance. The Automated Neuropsychological Assessment Metrics, version 4 (ANAM4), was used before and four times during each dive — with three intermittent periods of mild or moderate exercise during each dive — to measure nine cognitive domains. No dose-related effect of $CO_2$ was found. Basic cognitive domains of simple reaction time, visual scanning, visuo-spatial processing, and learning were unaffected, while fatigue and the higher cognitive functions of short-term memory (STM), long-term memory (LTM), working memory (WM), math processing, and sustained attention produced perplexing results. Most consistent of all differences was the decrease in LTM while divers were on $CO_2$, a decrease that persisted in Phase 1 even after divers were removed from $CO_2$ and returned to $O_2$. Math processing, WM, and sustained attention increased among divers both during and after breathing $CO_2$. STM decreased on $CO_2$ in Phase 1 but not in Phase 2. No cognitive changes were detected on air, when end tidal $CO_2$ remained closer to normal than on $O_2$. While some participants reported mild to moderate symptoms (e.g., headache, shortness of breath, irritability, and lack of concentration), end tidal $CO_2$ levels were mostly <7% Surface Equivalent Value (SEV). Because subjects were not hypercapnic, we cannot address the question of the study. Further investigation of the effects of inspired $CO_2$ on higher cognitive domains — along with consideration of breathing resistance — is recommended.


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## INTRODUCTION

Hypercapnia (elevated arterial carbon dioxide partial pressure [$P_aCO_2$]) represents a potential hazard for Navy divers who use closed- or semiclosed-circuit underwater breathing apparatus (UBAs), because premature and undetected scrubber breakdowns can cause them to inhale high levels of $CO_2$. Scrubber duration times for closed or semiclosed rebreather UBAs are measured from test start to the time that effluent $CO_2$ reaches 0.5% surface equivalent value (SEV). However, it has been proposed to increase duration times by easing $CO_2$ limits to 2% SEV.[1] Working Navy divers may then be at increased risk of hypercapnia.

$P_aCO_2$ may increase when subjects inhale $CO_2$. $CO_2$ retention may also result from psychological sources such as anxiety,[2] or because excessive effort is needed to move gas, arterial chemoreceptors are insensitive to $CO_2$, or the regulatory systems balance the metabolic costs of increased breathing against those of moderate $CO_2$ accumulation. Work of breathing may become excessive with increased gas density at great depth,[3] with high breathing resistance (in certain masks), or with other loads (e.g., pressure imbalances, elastic load, exercise) on breathing. In submerged working divers, several of these factors may occur simultaneously, and dry studies (performed in air vice water) perhaps give a false sense of the safety in moderate inspired fractions of $CO_2$.

Symptoms of hypercapnia include confusion, inability to concentrate, drowsiness, and loss of consciousness.[4] Relaxed scrubber duration criteria may also increase the risk of central nervous system (CNS) oxygen toxicity at oxygen partial pressures ($P_{O_2}$) where this toxicity does not normally occur.[2,5] However, CNS toxicity is not the focus of our study, and we address it only in our provisions for subject safety.

Dry exercise in conjunction with inhaled $CO_2$ has been shown to increase $P_aCO_2$.[6] Dry exercise alone has been shown to increase $P_aCO_2$ for some divers more than for nondivers.[7]

To our knowledge, effects of elevated inspired $CO_2$ on cognitive performance during submerged exercise have not been scientifically explored. Mixed results have been reported from dry studies,[2,3,5] two of which[3,5] showed effects during exposure and one[2] only during recovery. Elevated end tidal $CO_2$ ($F_{ET}CO_2$, which is related to $P_aCO_2$) of 7% or 8% was associated with decrements in cognitive performance.[3,5] In one study, little cognitive or behavioral effect was measureable until end tidal partial pressure $CO_2$ (end tidal $P_{CO_2}$) exceeded 51 Torr (equivalent to 7% SEV $CO_2$),[5] when performance on logical and mathematical reasoning tasks was significantly slowed — while accuracy in logical reasoning tasks, short- and long-term memory, and alertness remained unaffected. The other study[3] showed no performance changes at 4% or 6.6% $F_{ET}CO_2$. However, at $F_{ET}CO_2$ of 8% subjects showed significant cognitive and psychomotor decrements. Another dry study showed performance decrements only during recovery from breathing 6% $CO_2$,[2] decrements that were associated with decreases in $P_{CO_2}$ from baseline after $CO_2$ breathing.

The current study, conducted under Naval Sea Systems Command (NAVSEA) Task 09-01,[8] addresses effects of inspired $CO_2$ on the cognitive function of submerged working divers. Effects are measured under very mildly hyperoxic ($PO_2$ = 0.3 atmospheres [atm]) and hyperoxic ($PO_2$ = 1.4 atm) conditions, because hyperoxia itself causes some central hypercapnia.[9] $CO_2$ levels that may be encountered during working dives, 1.5% and 3% SEV, are used. The goals of the study are to measure the cognitive effects of hypercapnia, specifically to (1) evaluate how inhaled $CO_2$ in oxygen cognitively affects submerged working divers, (2) determine whether switching to inspired gas free of $CO_2$ results in a further change in performance or to a restoration to baseline, and (3) evaluate differences in how $CO_2$ effects might be related to $PO_2$.

# METHODS

## GENERAL

Diver subjects were active-duty Navy diving personnel recruited from the Navy Experimental Diving Unit (NEDU) and from other diving commands by E-mail and word of mouth. All were male, and all gave their written informed consent.

Approved by the NEDU Institutional Review Board and conducted under Protocol 09-04/32220 and BUMED number NEDU.2009.0005, this study evaluated the cognitive performance of divers who, submerged and exercising in the NEDU test pool at chest depth of about 12 feet of water, breathed 0%, 1.5%, or 3% $CO_2$ SEV, either in oxygen ($PO_2$ = 1.4 atm) or in air ($PO_2$ = 0.3 atm). Water temperature was 82 ± 5 °F, and divers, dressed for comfort, breathed either humidified, open circuit, surface-supplied $O_2$ (Phases 1 and 2; $PO_2$ approximately 1.4 atm) or air (Phase 3; $PO_2$ approximately 0.3 atm), with or without added $CO_2$. Pairs of divers breathed from the same gas supply. In addition to the usual dive-side team, a standby diver was present on the pool deck whenever divers were in the water.

All dives lasted for three and one-half hours. Beginning and ending with rest periods, divers alternated between 30-minute rest periods and 30-minute periods of cycle ergometer exercise. During all rest periods, they completed computerized cognitive tests. The first 30-minute rest period, without inspired $CO_2$ and before exercise, provided the in-water (“wet”) baseline.

Gas mixtures were prepared in advance, and divers were not told the $CO_2$ fraction that they were breathing. To mimic swimming, exercise intensity was mild (Phase 1) or moderate (Phases 2 and 3).

## EXPERIMENTAL DESIGN AND ANALYSIS

Cognitive variables

Cognitive function was measured with sections of the Automated Neuropsychological Assessment Metrics, version 4 (ANAM4),[10] which has been used to assess divers' cognitive performance under many conditions.[10–15] With a library of tests designed to evaluate a broad spectrum of clinical and research applications, the ANAM4 is a computer-based assessment battery developed by the Department of Defense. It consists of 11 subtests, nine of which were used in this protocol — specifically, the Stanford Sleepiness Scale (SS), Simple Reaction Time (SRT), Code Substitution (CDS), Code Substitution with Delay (CDD), Matching (MTG), Matching to Sample (M2S), Mathematical Processing (MTH), Sternberg Memory Search (ST4), and Running Memory Continuous Performance Test (CPT). We chose to exclude the mood scale and logical reasoning tests. A description of each included subtest follows.

Stanford Sleepiness Scale (SS). This scale consists of seven statements that describe how one feels with respect to alertness or sleepiness. It has been designed to provide a state or trait assessment of energy-fatigue level.[10]

Simple Reaction Time (SRT). The ANAM4 version of SRT serves two purposes: to measure pure reaction time or basic psychomotor speed, and to partial out the effects of motor or peripheral nerve conduction velocity times from actual cognitive processing time. This test presents a simple stimulus on the screen, and the participant is instructed to press a response key each time the stimulus is presented.[10]

Code Substitution (CDS). In this test a key containing a string of up to nine symbols and nine digits is displayed across the upper portion of the screen. Symbols and numerals are paired, with a unique number located below a specific symbol. During the task, a "test" pair (i.e., a symbol and digit) is presented at the bottom of the screen, below the key containing the correct symbol number pairs. The objective is to identify whether the test pair matches the associated pair in the key at the top of the screen. Responses consist of pressing one of two specified mouse buttons. This test measures learning.[10]

Code Substitution with Delay (CDD). After the CDS learning trial, an associative recognition memory trial is presented. The procedure is similar to that of the CDS, but the key is not displayed. The participant indicates whether or not the displayed pair is correct on the basis of his or her recollection of the pairs presented during the learning trial. This test measures long-term memory (LTM).[10]

Matching Grids (MTG). This task measures visual scanning by requiring the participant to match two 4 x 4 matrix (checkerboard) patterns that are presented side-by-side and in the same orientation.[16]

Matching to Sample (M2S). In this test the participant is required to match a block pattern from memory. A single 4 x 4 checkerboard matrix is presented in the center of the screen as a sample stimulus. For each trial presentation of a matrix, the number of cells that are shaded varies at random. Following a prespecified time interval — in our case, 5 seconds — two comparison matrices are presented side by side. One matches the sample matrix, while the other differs in one cell. The participant's task is to indicate which matrix matches the sample matrix. This is a test of visuo-spatial processing.[16]

Sternberg Memory Search (ST4). This ANAM4 adaptation of the Sternberg serial reaction time paradigm requires participants to memorize a string of four letters. The string disappears from view after 5 seconds, and individual letters are presented one at a time. The participant's task is to decide whether the letter presented belongs or does not belong to the string. This is a test of short-term memory (STM).[16]

Mathematical Processing (MTH). During this task measuring mathematical processing, arithmetic problems are presented in the middle of the screen. The task involves deducing an answer and then determining whether that answer is greater or less than the number 5. Each problem includes two mathematical operations (addition and subtraction) on sets of three one-digit numbers (e.g., $5 + 3 – 4 = ?$). The participant is instructed to indicate whether the answer is greater than or less than 5 by pressing one of two specified response buttons.[10]

Running Memory Continuous Performance Test (CPT). This continuous number comparison test asks the participant to monitor a randomized sequence of single-digit numbers presented one at a time in the center of the screen. Participants are to press a specified key if the digit on the screen matches the digit that has immediately preceded it or to press a different key if the digit does not match. This test measures working memory (WM) and sustained attention.[10]

Serial assessment is conventionally employed to aid decisions regarding change in cognitive status. The significance of any cognitive change observed may be obscured by practice effects, which act to enhance performance following repeated exposure to the test. However, the positive effects of practice are most evident between the first and second administration of a cognitive test, with performance stabilizing between second and subsequent assessments.[17] To account for training effects, divers completed the test battery four times before their dive days. The first two of these practice tests were not used in data analysis. The last two were averaged to provide a "dry" baseline score.

Changes in cognitive dependent variables were assessed by comparing throughputs, the numbers of successes per unit of time.[18] Throughput scores were recorded for all subtests except SS, for which participant scores were simply recorded as the given response on the scale of 1–7.

Repeated measures analysis of variance (ANOVA) was used to analyze the ANAM4 in-water results before and after exercise. When the ANOVA indicated an overall effect, the Bonferonni correction was used to make pairwise comparisons. Paired t-tests of wet and dry baselines were used to assess the effects of immersion.

All phases provided comparisons between dry and wet baselines. Phase 1 was planned to separate the effects of exercise alone, the acute effects of inspired $CO_2$, and the aftereffects of inspired $CO_2$ in a repeated measures design — and to measure dose effects of $CO_2$ as a between-subject variable (Table 1). By presenting the three gas conditions in all possible combinations (Table 2), Phase 2 was designed to investigate the effects of order of gas presentation and to control for fatigue or familiarization with the tests. Phase 3, which matched Group 4 from Phase 2 and was designed, in conjunction with Phase 2, was to assess the effect of $P_{O_2}$ on any of the variables (Table 3). For analysis of the effects of $P_{O_2}$, Phase 3 was compared to Group 4 of Phase 2 in a repeated-measures ANOVA with background gas as a between-subject variable.

Table 1. Inspired $CO_2$ fraction (SEV) and exercise periods, Phase 1.

| **Stages** | **30 min** | **30 min** | **30 min** | **30 min** | **30 min** | **30 min** | **30 min** |
|---|---|---|---|---|---|---|---|
| | Rest ANAM | Bike | Rest ANAM | Bike | Rest ANAM | Bike | Rest ANAM |
| Group a (n = 20) | $O_2$ | $O_2$ | $O_2$ | 1.5% $CO_2$ | 1.5% $CO_2$ | $O_2$ | $O_2$ |
| Group b (n = 16, 20 planned) | $O_2$ | $O_2$ | $O_2$ | 3% $CO_2$ | 3% $CO_2$ | $O_2$ | $O_2$ |

Table 2. Inspired $CO_2$ fraction (SEV) and exercise periods, Phase 2.

| **Stages** | **30 min** | **30 min** | **30 min** | **30 min** | **30 min** | **30 min** | 30 min |
|---|---|---|---|---|---|---|---|
| | Rest ANAM | Bike | Rest ANAM | Bike | Rest ANAM | Bike | Rest ANAM |
| Group 1 (n = 6) | $O_2$ | $O_2$ | $O_2$ | 1.5% $CO_2$ | 1.5% $CO_2$ | 3% $CO_2$ | 3% $CO_2$ |
| Group 2 (n = 6) | $O_2$ | $O_2$ | $O_2$ | 3% $CO_2$ | 3% $CO_2$ | 1.5% $CO_2$ | 1.5% $CO_2$ |
| Group 3 (n = 6) | $O_2$ | 1.5% $CO_2$ | 1.5% $CO_2$ | $O_2$ | $O_2$ | 3% $CO_2$ | ≤3 $CO_2$ |
| Group 4 (n = 6) | $O_2$ | 1.5% $CO_2$ | 1.5% $CO_2$ | 3% $CO_2$ | 3% $CO_2$ | $O_2$ | $O_2$ |
| Group 5 (n = 6) | $O_2$ | 3% $CO_2$ | 3% $CO_2$ | $O_2$ | $O_2$ | 1.5% $CO_2$ | 1.5% $CO_2$ |
| Group 6 (n = 4, 6 planned) | $O_2$ | 3% $CO_2$ | 3% $CO_2$ | 1.5% $CO_2$ | 1.5% $CO_2$ | $O_2$ | $O_2$ |

Table 3. Inspired $CO_2$ fraction (SEV) and exercise periods, Phase 3.

| Stages | 30 min | 30 min | 30 min | 30 min | 30 min | 30 min | 30 min |
|---|---|---|---|---|---|---|---|
| | Rest ANAM | Bike | Rest ANAM | Bike | Rest ANAM | Bike | Rest ANAM |
| Group 1 (n = 16) | Air | 1.5% $CO_2$ in air | 1.5% $CO_2$ in air | 3% $CO_2$ in air | 3% $CO_2$ in air | Air | Air |
| Group $4_2$ * (n = 6) | $O_2$ | *1.5% $CO_2$* | *1.5% $CO_2$* | *3.0% $CO_2$* | *3.0% $CO_2$* | *$O_2$* | *$O_2$* |

*****Only Group 1 was tested in Phase 3. Group $4_2$, in italics, is Group 4 from Phase 2, to compare effects of air versus $O_2$.

End Tidal $CO_2$ Fraction

During testing, $F_{ET}CO_2$ was monitored as a potential independent variable in the analysis of cognitive function. End tidal values were recorded both manually and electronically. For Phase 1a (1.5% $CO_2$) and Phase 3, local maxima were read and averaged from the computer record. Respiratory frequency also was calculated from the average period of the selected three to ten breaths. Because the processing was very slow, the end tidal values recorded on paper during the other phases of the study were used instead, and breathing frequency was not estimated.

Repeated measures ANOVA with contrasts was used to compare $F_{ET}CO_2$ across gases and exercise condition.

Pulmonary Function Tests (PFTs)

Three reproducible flow-volume loops were recorded each time pulmonary function was measured, and the averages of the three were reported for forced vital capacity (FVC), forced expired volume in one second ($FEV_1$), average forced expired flow between 25% and 75% of volume expired ($FEF_{25–75}$), and peak forced expired flow ($FEF_{max}$). Values measured within one hour of surfacing and on the first day after the dives were compared to those for the same subject measured before diving (baseline). When PFTs were conducted, subjects were asked about symptoms of pulmonary oxygen toxicity.

Values were considered low if they were outside the lower limits of normal variability previously determined at NEDU[19] — namely, 7.7% for FVC, 8.4% for $FEV_1$, 16.8% for $FEF_{25–75}$, and 17% for $FEF_{max}$. We assumed that the small amount of $CO_2$ would not affect pulmonary function, and we hypothesized that 3.5-hour dives with exercise would be indistinguishable from 4-hour dives at rest. The incidence for all these parameters of measurable changes after diving was compared to that for previous 4-hour dives.

## EQUIPMENT AND INSTRUMENTATION

Gas Supply

Two oxygen-regulating console assemblies (ORCAs) — manifolds designed to supply up to three divers at depths up to 30 feet of seawater (fsw) — were used to supply gas to four divers, one ORCA for each dive pair. It takes gas from three sources — diver's air, high pressure (HP) $O_2$, and low pressure (LP) $O_2$ — and allows switching among these sources. For this study, air and LP $O_2$ were supplied from the test pool console, and the two HP $O_2$ ports were connected to two K bottles, one containing 1.1% $CO_2$ in $O_2$ and one containing 2.2% $CO_2$ in $O_2$. (For Phase 3, the K bottles contained $CO_2$ in air.)

Gas was bubbled through water to gain humidity on its way to MK 20 masks (Interspiro; Cliffwood Beach, NJ) worn by the divers.

Ergometer Exercise

Exercise was imposed on underwater cycle ergometers assembled at NEDU. The pedals drive the shaft of a hysteresis brake (Magtrol, HB210; Buffalo, NY) through a gear train with an overall gear ratio of 1:19.2. The torque necessary to turn the brake is regulated by the electric current supplied to the brake. The ergometers are calibrated dry at 60 rotations per minute (rpm). Since power is proportional to the product of torque and rotational speed, a cyclist can decrease total power output from the nominal setting by pedaling more slowly and can increase power expended by pedaling faster. Cycling in the water adds a significant load to that of the brake: about 50 W at 60 rpm — more at a higher cadence, and less at a lower cadence.[20]

In Phase 1, ergometers were set to 25 W as calibrated at 60 rpm, a load corresponding on the average to 75 W at 60 rpm in the water,[20] but pedal cadence was not controlled. In Phases 2 and 3, relative exercise intensity was determined by diver heart rates, with ergometer loads adjusted to maintain heart rates of 105 ± 5 beats per minute. Ergometers were set to 50 W as calibrated at 60 rpm, and divers, who wore chest-strap heart rate monitors with wrist displays (Polar Electro; Woodbury, NY), were asked to adjust pedal cadence for the target heart rate and to call for ergometer adjustment if it was needed.

Underwater ANAM4[21]

Each of the two pieces of underwater ANAM4 testing equipment consisted of a topside laptop computer, a topside control box, and an underwater keypad and monitor. The ANAM4 software was installed on the computer. A USB/Ethernet adaptor-insulated cable plugged into the USB port of the laptop connected the laptop with the underwater keypad. Two converter boxes on this cable converted the USB signal from the laptop into Ethernet format for long-distance transmission and then reconverted this signal at the keypad. The laptop–underwater monitor interface used a video graphics array cable plugged on one end into the back of the laptop and on the other end into the topside control box. An insulated Ethernet cable connected the topside control box to the underwater monitor and allowed the laptop screen to be projected on the underwater monitor.

Topside personnel entered the diver's assigned ID into the ANAM4 test screen on the laptop. At 30-minute intervals two submerged divers were instructed to report to the testing station and begin the cognitive test, while the other two divers moved to the underwater cycle ergometers to begin exercise. Each complete test session lasted approximately 20 minutes. The underwater monitor displayed the test questions, and the diver used the keypad to respond to the test stimuli. The Principal Investigator (PI) viewed the responses on the topside laptop in real time, and these were stored in a laptop computer file for subsequent analysis.

Pulmonary Function Measurements

Forced flow-volume parameters were collected with a volume-based spirometer system (CPL, nSpire Health; Longmount, CO). Three consistent flow-volume loops were recorded each time pulmonary function was measured, and the averages of the three were reported.

End Tidal $CO_2$ Monitoring

To permit measurement of exhaled $CO_2$, five masks (four for divers, plus one spare) were fitted with faceplates drilled on the left side to receive 1/8” Nylaflow® tubing (S & L Plastics; Nazareth, PA) that penetrated the oronasal cup and terminated just below the nose block.

Laminar flow calculations indicated that a hydrostatic pressure of 9.8 fsw (30 kPa) would drive 113 mL/s through 100 ft of 1/8” internal diameter tubing, and 150 mL/s through 75 ft of the same tubing. Calculated mean transit times were 2.1 s for 100 ft and 1.2 s for 75 ft. With 100 ft of tubing, gas composition measurements of normal breathing showed a distinct breath pattern: a return to 0% $CO_2$ on inspiration, but with slightly attenuated expiratory peaks and indications of some mixing in the line. Gas flow was not measured.

We required only 75 ft of tubing. To reduce the risk of entanglement, about 30 ft of the tubing from each mask was tied to its breathing and communications umbilical. From the umbilicals, the free 45 ft of tubing was bundled and brought to the window of the data acquisition area, where each end was labeled by diver (red, green, yellow, or blue), terminated with a Swagelock (Solon, OH) connector, and capped. A sector mass spectrometer (Marquette MGA 1100, Marquette Gas Analysis; St. Louis, MO) in the data acquisition area was used to sample gas from the lines. A data acquisition computer running LabVIEW (National Instruments, Austin, TX) was used to display and store the mass spectrometer $CO_2$ signal.

We used a plexiglas half-cylinder block as the interface between the diver sample lines and the mass spectrometer sample line. The block was drilled axially with interior bore 1/8” and supplied with Swagelock fittings at each end to connect to the Nylaflow tubing, and the mass spectrometer sample line was inserted through a radial hole into the center of the bore. The mass spectrometer draws a 60 mL/min sample. When the line from a diver was connected to one end of the cylinder, the gas flowed through the sampling block and out through another 25 ft of small-bore, spirally-wound tubing into the room. Gas volumes greater than 60 mL/min flowed past the mass spectrometer probe, but room air was not drawn into the line if the flow was instantaneously low. We watched the plexiglas for any signs of water incursion and removed the mass spectrometer probe if it seemed prudent; water block filters degraded the gas step response unacceptably for end tidal sampling.

When expired $CO_2$ was to be measured for a diver, the sample line from that diver was uncapped and connected to the sample block. Data were collected for six to ten breaths before the line was disconnected and recapped, and another diver's $CO_2$ was measured.

## PROCEDURES

### Predive

Briefed about the experiment, the divers completed a document of informed consent and were assigned numerical identifiers before they began testing in any phase. All divers completed four ANAM4 practice sessions before diving each phase. If a diver did not understand the directions provided by the automated battery, the PI or the task leader was available to assist. For all subjects in Phases 1 and 2, and four of 16 subjects in Phase 3, the required ANAM4 practice tests were spread over a minimum of one and a maximum of 30 days. However, due to technical difficulties, 12 of 16 subjects in Phase 3 had a delay of up to 110 days between their practice and dive days.

Table 4. Symptoms queried during dives.

| | |
|---|---|
| Twitching or tingling | **Possible CNS – abort** |
| Visual disturbance | |
| Nausea | |
| Light-headedness or dizziness; i.e., "vertigo" | |
| Disorientation or irritability | |
| Changes in hearing | |
| Inspiratory burning | **Pulmonary** |
| Shortness of breath | |
| Rapid, shallow breathing | |
| Chest tightness | |
| Cough | |
| Difficulty breathing enough | **$CO_2$** |
| Hyperventilation | |
| Headache | |

### Dives

Divers reported to the physiology laboratory and completed a set of three technically acceptable PFTs. After a dive brief and under direction of the dive supervisor, the first pair of divers donned rigs and entered the test pool. The second dive pair followed 30 minutes later. Tables 1–3 list the gas conditions for all phases. $F_{ET}CO_2$ from each diver was read approximately every five minutes. While divers were underwater, they were asked about specific symptoms of pulmonary toxicity, CNS oxygen toxicity, and hypercapnia (Table 4). Questions were asked every 30 minutes, at the change from rest to exercise or vice versa. Divers were instructed to report severe symptoms of any kind and even mild CNS oxygen toxicity symptoms at any time. Some symptoms tabulated under "Possible CNS" were also possible $CO_2$ symptoms, and the response to those reports depended on corpsman and investigator judgments.

## RESULTS

A total of 86 dives were conducted through three phases of study (Phase 1a = 20, Phase 1b = 16, Phase 2 = 34, Phase 3 = 16). Subjects consisted of 45 male Navy divers. Thirty subjects participated in only one phase (Phase 1a = 5, Phase 1b = 4, Phase 2 = 16, Phase 3 = 5). The remaining 15 subjects participated in two or more phases.

<u>End Tidal $CO_2$</u>

End tidal values cannot be considered precise, since we did not attempt to optimize the sample line diameter of the gas sampling system for response time. However, the system provided breath-by-breath patterns with clearly distinguishable alveolar plateaus and reasonable baseline values (Figure 1).

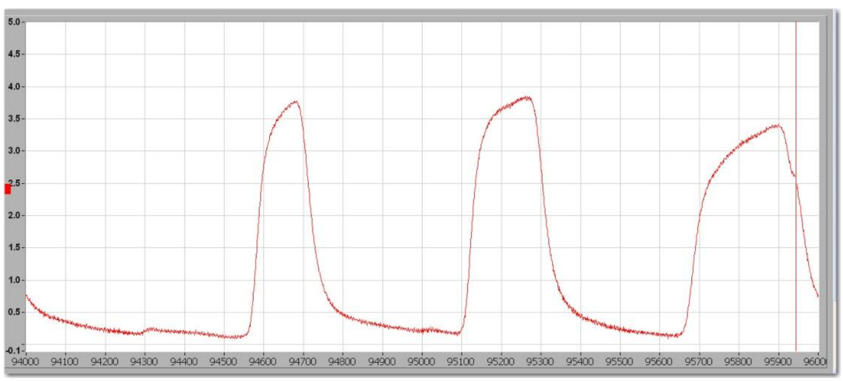


**Figure 1.** Sample $CO_2$ tracing from the bottom of the test pool, resting subject breathing air. The values on the y-axis show $CO_2$ percentages on the bottom; for SEV, multiply by 1.35. $F_{ET}CO_2$ = 5% SEV in the first two breaths and 4.6% SEV in the third.

### PHASE 1

<u>End Tidal $F_{CO_2}$</u>

Mean $F_{ET}CO_2$, thus $P_aCO_2$, was higher with 3% SEV $CO_2$ inhaled than with 1.5% SEV $CO_2$ inhaled (Table 5). At rest, $F_{ET}CO_2$ with 1.5% SEV $CO_2$ was maintained at baseline. Although the exercise period with no $CO_2$ following an hour with 1.5% $CO_2$ ("No $CO_2$ 2") had higher $F_{ET}CO_2$ than that before administration of $CO_2$ ("No $CO_2$ 1"), no other residual or "off" effect in $F_{ET}CO_2$ was evident.

$F_{ET}CO_2$ greater than 50 Torr (7% SEV) was measured in one subject during exercise with 1.5% SEV inspired $CO_2$, and in four subjects during exercise with 3% SEV inspired $CO_2$. The highest $F_{ET}CO_2$ was 8.1% SEV. The subject whose exercise $F_{ET}CO_2$ with inspired 1.5% $CO_2$ was elevated — 8.0% SEV — also retained $CO_2$ during exercise without $CO_2$ after the $CO_2$-breathing period, when his $F_{ET}CO_2$ was 7.1%. No resting $F_{ET}CO_2$ reached 7% SEV in Phase 1.

Table 5. $F_{ET}CO_2$ from Phase 1, mean and standard deviation (% SEV).

| Phase 1 | Exercise | | | Postexercise rest | | |
|---|---|---|---|---|---|---|
| | No $CO_2$ | | $CO_2$ | No $CO_2$ | | $CO_2$ |
| Inspired $CO_2$ | 1 | 2 | | 1 | 2 | |
| (a) 1.5% n = 20 | 4.8 (0.6) | 5.6 (0.6) | 6.0 (0.6) | 5.0 (0.6) | 5.0 (0.6) | 5.2 (0.5) |
| (b) 3% n = 16 | 5.1 (0.6) | 5.2 (0.7) | 6.7 (0.8) | 4.7 (0.6) | 5.0 (0.7) | 5.4 (0.5) |

Overall, $F_{ET}CO_2$ during exercise was significantly different ($p<0.01$) from that at rest. During exercise, $F_{ET}CO_2$ with either 1.5% or 3% inspired $CO_2$ was significantly greater ($p<0.01$) than that before $CO_2$ was inhaled (Table 5, "No $CO_2$ 1"). However, when postexercise resting values were recorded, $F_{ET}CO_2$ had not recovered fully after inspiration of 1.5% SEV $CO_2$ — "No $CO_2$ 2" and "No $CO_2$ 1" differed ($p<0.01$), but $F_{ET}CO_2$ had recovered after inspiration of 3% CO. "No $CO_2$ 1" and "No $CO_2$ 2" did not differ ($p>0.06$). At rest, $F_{ET}CO_2$ did not change ($p>0.1$) when 1.5% SEV $CO_2$ was inhaled, but it increased ($p<0.01$) when 3% SEV $CO_2$ was inhaled.

Cognitive Testing

Although the plan was to test 20 divers in each group, only 16 were able to complete Phase 1b, because one of the underwater computer monitors failed. Sizes of the main post hoc effects for the nine cognitive subtests turned out to be small to moderate, at best. Two-tailed post hoc power analysis for small (0.2) and moderate (0.5) effect sizes (alpha = 0.05, sample size = 36) showed experimental powers of 0.084 and 0.31, respectively.

Paired-sample t-tests between dry and wet baselines revealed differences in two subtests: SS ($t = 3.61$, $df = 35$, $p<0.01$), and SRT ($t = 3.86$, $df = 35$, $p<0.01$) [Table 6]. Subjects reported less fatigue and were slower to respond in water than they were in the dry baseline.

Table 6. Phase 1 dry and wet baseline means, SDs, and SEMs.

| Cognitive Subtest | n = 36 | Mean | SD | SEM |
|---|---|---|---|---|
| SS<br>(t = 3.61, p<0.01) | Dry Baseline<br>Wet Baseline | 2.2<br>1.8 | 1.0<br>0.8 | 0.2<br>0.1 |
| SRT (#Correct/min)<br>(t = 3.86, p<0.01) | Dry Baseline<br>Wet Baseline | 221<br>193 | 36<br>27 | 6<br>4 |
| CDS (#Correct/min)<br>(t = –0.77, p>0.4) | Dry Baseline<br>Wet Baseline | 48<br>49 | 10<br>8 | 2<br>1 |
| MTG (#Correct/min)<br>(t = –1.12, p>0.2) | Dry Baseline<br>Wet Baseline | 37<br>38 | 7<br>7 | 1<br>1 |
| M2S (#Correct/min)<br>(t = 0.41, p>0.6) | Dry Baseline<br>Wet Baseline | 36<br>36 | 9<br>8 | 2<br>1 |
| CDD (#Correct/min)<br>(t = –1.52, p>0.1) | Dry Baseline<br>Wet Baseline | 43<br>45 | 11<br>11 | 2<br>2 |
| ST4 (#Correct/min)<br>(t = –0.43, p>0.6) | Dry Baseline<br>Wet Baseline | 84<br>85 | 14<br>14 | 2<br>2 |
| MTH (#Correct/min)<br>(t = –1.15, p>0.2) | Dry Baseline<br>Wet Baseline | 26<br>27 | 6<br>6 | 1<br>1 |
| CPT (#Correct/min)<br>(t = 0.84, p>0.4) | Dry Baseline<br>Wet Baseline | 102<br>99 | 20<br>13 | 3<br>2 |

Unit for SS is response on a scale of 1–7. All other units are for throughput, number correct /minute. Subtests with no significant difference, wet to dry, are shaded.

Separate repeated measures analysis of variance (ANOVA) for each of the nine cognitive domains revealed significant differences across ANAM4 tests 1, 2, 3, and 4 within subjects in four of the nine subtests — CDD, ST4, MTH, and CPT — but no difference (p>0.05) for five of the nine cognitive subtests — SS, SRT, CDS, MTG, and M2S. No differences (p>0.05) were found between Group a (1.5% $CO_2$) and Group b (3% $CO_2$) for any of the nine cognitive domains assessed.

*Hindered Performance on $CO_2$*

CODE SUBSTITUTIONS WITH DELAY (CDD). Maulchy's test (a conventional test used to validate repeated-measures factor ANOVAs) indicated that the assumption of sphericity had not been violated (chi-square = 6.634, p=0.249). The results show a significant main effect of test — that is, in-water ANAM4 numbers 1–4 — on LTM [$F$ (3, 102) = 7.563, p<0.001]. Pairwise analyses revealed that LTM was significantly lower on $CO_2$ (p<0.05) or on the second $O_2$ period (p<0.05) ($O_2$-2 on Figures 2–5) than it was at wet baseline; CDD throughput decreased almost eight points from baseline. When only the postexercise values were compared to one another (Figure 2), CDD throughput on $CO_2$ (Test 3) was statistically lower than that on $O_2$ postexercise (Test 2).

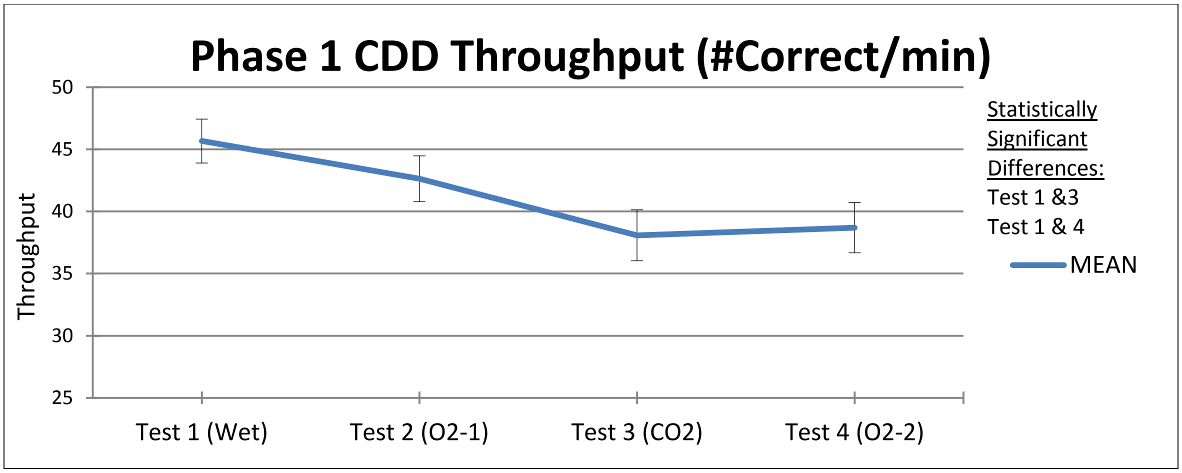


**Figure 2.** Phase 1 CDD throughput (#correct/min). Error bars represent SEM.

STEINBERG MEMORY SEARCH (ST4). Because Maulchy's test indicated that the assumption of sphericity had been violated (chi-square = 17.947, p=0.003), degrees of freedom were corrected with Greenhouse-Geisser estimates of sphericity ($\varepsilon$ = 0.742). Repeated measures ANOVA shows a significant main effect of test on ST4 throughput, a measure of STM [$F$ (3, 102) = 3.164, p=0.043]. Pairwise analyses reveal that ST4 throughput was significantly lower on $CO_2$ than on $O_2$-1 ($p<0.05$), though it was not significantly decreased from wet baseline. Throughput from ST4 after exercise was seven points lower with $CO_2$ than with 100% $O_2$ before or after the $CO_2$ (Figure 3).

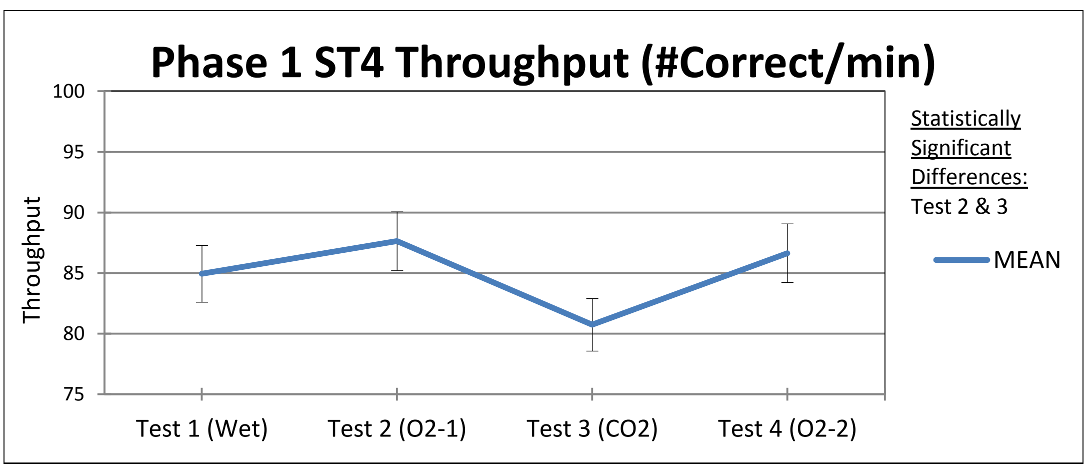


**Figure 3.** Phase 1 ST4 throughput (#correct/min). Error bars represent SEM.

*Enhanced Performance upon Return to $O_2$*

MATH PROCESSING (MTH). Because Maulchy's test indicated that the assumption of sphericity had been violated (chi-square = 21.648, p=0.001), degrees of freedom were corrected with Greenhouse-Geisser estimates of sphericity (ε = 0.690). Repeated measures ANOVA shows a significant main effect of test on math processing [*F* (3, 102) = 3.695, p=0.028]. Pairwise analyses revealed that MTH was significantly higher on $O_2$-2 than on $O_2$-1 (p<0.05); math processing throughput did not differ from baseline until the final $O_2$ period, when it was 2.5 points higher (Figure 4).

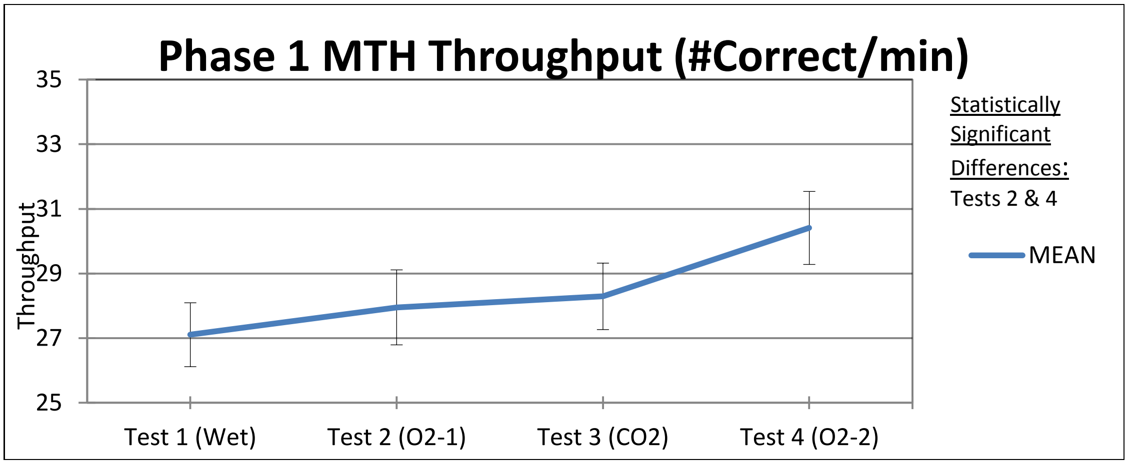


**Figure 4.** Phase 1 MTH throughput (#correct/min). Error bars represent SEM.

CONTINUOUS PERFORMANCE TEST (CPT). Maulchy's test indicated that the assumption of sphericity had not been violated (chi-square = 6.392, p=0.27). Repeated measures ANOVA shows a significant main effect of test on CPT, a measure of WM and sustained attention [*F* (3, 102) = 3.981, p=0.010]. Pairwise analyses revealed that WM and sustained attention were significantly higher on $O_2$-2 than on $O_2$-1 (p<0.05); CPT throughput trended up through the $CO_2$ phase to the final $O_2$ phase, with a significant difference of seven points between $O_2$ phases (Figure 5).

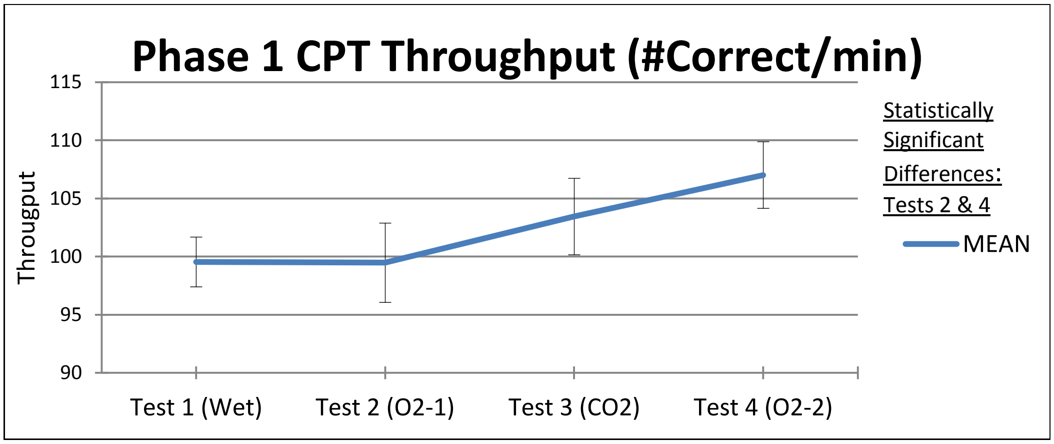


**Figure 5.** Phase 1 CPT throughput (#correct/min). Error bars represent SEM.

Symptoms Related to $CO_2$ Breathing

Three of the Phase 1 subjects who breathed 1.5% SEV $CO_2$ (Phase 1a) and eight of those who breathed 3% $CO_2$ (Phase 1b) reported one or more symptoms probably related to inhaled $CO_2$ (Table 4). No relation was evident between reported symptoms and $F_{ET}CO_2 \geq 7\%$ (Table 7), but a 3% inhaled $CO_2$ fraction appears to result in more reported symptoms than 1.5% inhaled $CO_2$ does.

Table 7. Symptoms of $CO_2$ breathing reported during or after Phase 1 dives. Subjects often reported multiple symptoms.

| **Phase 1a: 1.5% $CO_2$ inhaled in $O_2$** | n = 20 |
|---|---|
| **Symptom** | **Number reporting** |
| Headache | 3 |
| Shortness of breath | 1 |
| **Phase 1b: 3% $CO_2$ inhaled in $O_2$** | n = 16 |
| **Symptom** | **Number reporting** |
| Headache | 2 |
| Shortness of breath | 2 |
| Poor concentration | 2 |
| Irritability | 2 |
| Light-headed, altered mental state | 1 |
| Nausea | 1 |

Table 8. Contingency tables: Relation between number of Phase 1 subjects reporting symptoms and number retaining $CO_2$.

| **Phase 1a** | $F_{ET}CO_2$ ≥7% | $F_{ET}CO_2$ <7% | **Phase 1b** | $F_{ET}CO_2$ ≥7% | $F_{ET}CO_2$ <7% |
|---|---|---|---|---|---|
| Symptoms | 0 | 3 | Symptoms | 4 | 3 |
| No symptoms | 1 | 16 | No symptoms | 2 | 7 |

## PHASE 2

End Tidal $F_{CO_2}$

$F_{ET}CO_2$ during exercise with inspired $CO_2$ was elevated above that without $CO_2$ but did not differ between 1.5% and 3% SEV inspired $CO_2$. However, $F_{ET}CO_2$ at rest after exercise increased with increasing inspired $CO_2$ (Table 9).

Table 9. $F_{ET}CO_2$ from Phase 2, mean and standard deviation (% SEV).

| Phase 2 n = 34 | In-water Baseline | Exercise | | | Postexercise rest | | |
|---|---|---|---|---|---|---|---|
| Inspired $CO_2$ | 0% | 0% | 1.5% | 3% | 0% | 1.5% | 3% |
| Phase 2 %SEV | 5.0 (0.4) | 5.6 (0.7) | 6.2 (0.5) | 6.3 (0.8) | 4.9 (0.6) | 5.3 (0.6) | 5.7 (0.6) |

With no inspired $CO_2$, $F_{ET}CO_2$ at rest after exercise did not differ ($p>0.4$ by paired t-test) from that before exercise (baseline). $F_{ET}CO_2$ during exercise was significantly higher ($p<0.01$) than that at rest. During exercise, $F_{ET}CO_2$ was greater ($p<0.01$) with 1.5% $CO_2$ inspired than with no $CO_2$ inspired but did not differ ($p>0.3$) between 1.5% and 3% $CO_2$ inspired, while during postexercise rest, $F_{ET}CO_2$ increased ($p<0.01$) from 0% to 1.5% inspired $CO_2$ and from 1.5% to 3% inspired $CO_2$.

Ten subjects had $F_{ET}CO_2$ >7% during exercise in Phase 2: one with 0% inspired $CO_2$ (7.3% SEV), one with both 1.5% and 3% inspired $CO_2$ (7.2%, 7.4% SEV, respectively), and the other eight only with 3% inspired $CO_2$ (highest 7.6% SEV). Two Phase 2 subjects showed $F_{ET}CO_2$ = 7.0% while breathing 3% $CO_2$ at rest — that is, during ANAM4 testing.

Cognitive Testing

Although the plan was to have equal numbers in all groups, one subject in Group 6 was ill on the morning of his dive, and another diver aborted his dive for reasons discussed in the "PHASE 2 — Symptoms Related to $CO_2$ Breathing" subsection.

Paired-sample t-tests showed differences between dry and wet baseline in the cognitive subtests SS, SRT, CDS, M2S, ST4, and CPT (Table 10). For all subtests, df = 33 and alpha = 0.05.

Table 10. Phase 2 dry and wet baseline means, SDs, and SEMs.

| Cognitive Subtest | n = 34 | Mean | SD | SEM |
|---|---|---|---|---|
| SS<br>(t = 2.36, p<0.03) | Dry Baseline<br>Wet Baseline | 2.3<br>1.8 | 1.0<br>1.0 | 0.2<br>0.2 |
| SRT (#Correct/min)<br>(t = 10.53, p<0.01) | Dry Baseline<br>Wet Baseline | 253<br>183 | 23<br>37 | 4<br>6 |
| CDS (#Correct/min)<br>(t = 0.6, p>0.5) | Dry Baseline<br>Wet Baseline | 50<br>49 | 10<br>10 | 2<br>2 |
| MTG (#Correct/min)<br>(t = –1.23, p>0.2) | Dry Baseline<br>Wet Baseline | 38<br>39 | 10<br>9 | 2<br>2 |
| M2S (#Correct/min)<br>(t = 2.95, p<0.01) | Dry Baseline<br>Wet Baseline | 38<br>32 | 14<br>10 | 2<br>2 |
| CDD (#Correct/min)<br>(t = –2.17, p<0.04) | Dry Baseline<br>Wet Baseline | 46<br>50 | 14<br>14 | 2<br>2 |
| ST4 (#Correct/min)<br>(t = 2.41, p<0.03) | Dry Baseline<br>Wet Baseline | 89<br>83 | 18<br>17 | 3<br>3 |
| MTH (#Correct/min)<br>(t = 1.56, p>0.1) | Dry Baseline<br>Wet Baseline | 28<br>27 | 9<br>8 | 1<br>1 |
| CPT (#Correct/min)<br>(t = 4.12, p<0.01) | Dry Baseline<br>Wet Baseline | 112<br>100 | 29<br>25 | 5<br>4 |

Unit for SS is response on a scale of 1–7. All other units are for throughput, number correct/minute. Subtests with no significant difference, wet to dry, are shaded.

Repeated measures ANOVA on the between-subject factors showed no statistically significant differences among the six groups on any of the nine cognitive subtests. No main effect of order of presentation on cognitive performance (p>0.05) was evident. Repeated measures ANOVA within subjects effects for the four gas conditions (wet baseline $O_2$, $O_2$ after exercise, 1.5% $CO_2$ after exercise, and 3% $CO_2$ after exercise) revealed no statistically significant differences for the five cognitive subtests SRT, CDS, M, M2S, or ST4 (p>0.05). Differences were found on the four cognitive subtests SS, CDD, MTH, and CPT.

*Hindered Performance Compared to Wet Base*

Sleepiness scale (SS). Maulchy's test indicated that the assumption of sphericity had not been violated (chi-square = 7.427, p=0.191). Repeated measures ANOVA shows a significant main effect of gas on fatigue [$F$ (3, 84) = 6.117, p=0.001]. Pairwise analyses revealed that fatigue during all in-water tests after exercise was significantly greater than that during wet baseline (p<0.05) by 0.6 points on the scale. ANOVA across only the postexercise values (Tests 2–4) showed no difference with gas inhaled (Figure 6).

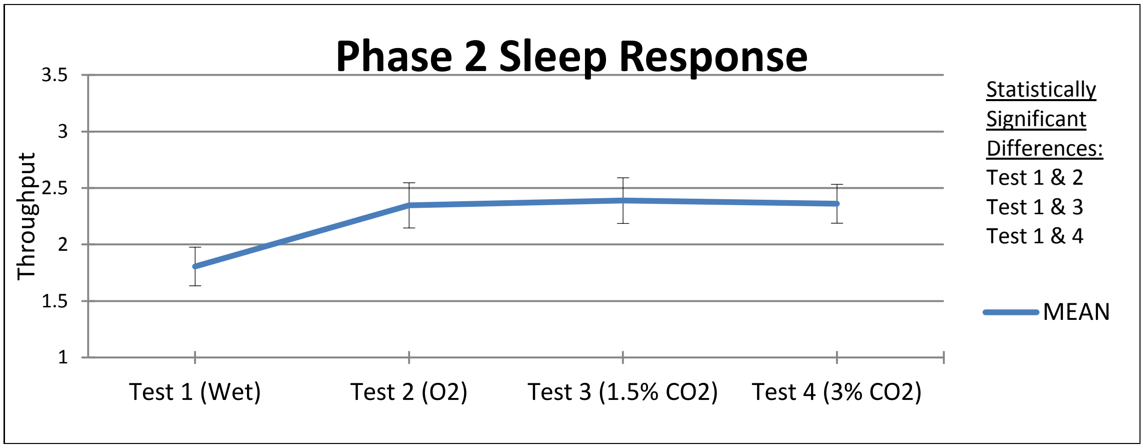


**Figure 6.** Phase 2 sleep response. Error bars represent SEM.

CODE SUBSTITUTIONS WITH DELAY (CDD). Maulchy's test indicated that the assumption of sphericity had not been violated (chi-square = 9.720, p=0.084). Repeated measures ANOVA shows a significant main effect of gas on CDD, a measure of LTM [$F$ (3, 84) = 6.115, p=0.001]. Pairwise analyses revealed that CDD throughput at wet baseline was significantly higher ($p<0.05$) than it was on 1.5% $CO_2$ or 3% $CO_2$. CDD throughput was reduced about 10 points when $CO_2$ was inhaled, with no $CO_2$ dose effect. With $O_2$ after exercise, CDD overall was not different from that at wet baseline ($O_2$ without exercise) (Figure 7). When only the CDD values after exercise (tests 2–4) were compared, the values with $CO_2$ were statistically lower than those without $CO_2$.

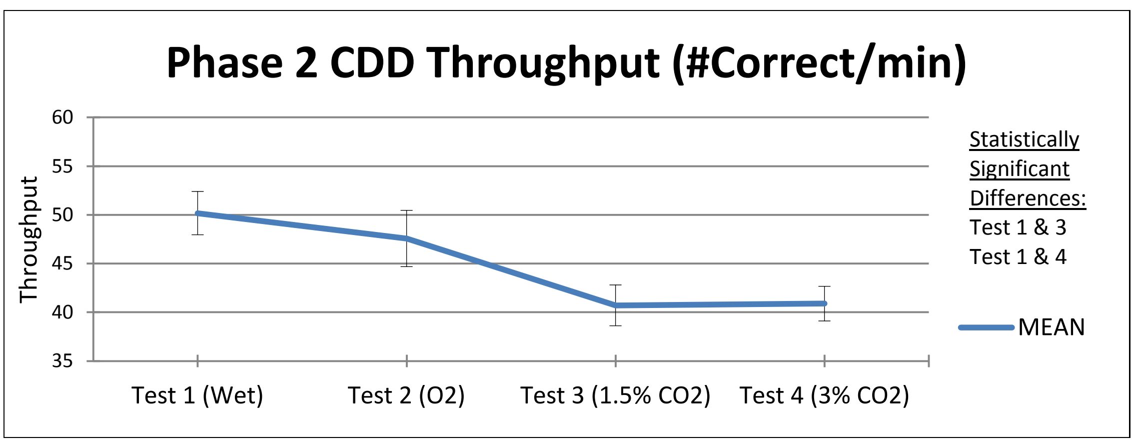


**Figure 7.** Phase 2 CDD throughput (#correct/min). Error bars represent SEM.

*Enhanced Performance Compared to Wet Base*

MATHEMATICAL PROCESSING (MTH). Maulchy's test indicated that the assumption of sphericity had not been violated (chi-square = 3.889, p=0.566). Repeated measures ANOVA showed a significant main effect of gas on MTH [$F$ (3, 84) = 5.867, p=0.001]. Pairwise analyses revealed that MTH throughput was significantly (p<0.05) lower than baseline on $O_2$ and on 1.5% $CO_2$, but not on 3% $CO_2$. Math processing throughput was three points higher than wet baseline with postexercise $O_2$, 2.8 points higher than baseline with 1.5% $CO_2$ postexercise, and 2.1 points higher than baseline (not statistically significant) with 3% $CO_2$ postexercise. The repeated measures ANOVA across only postexercise values (Tests 2–4) showed no differences of gas inhaled (Figure 8).

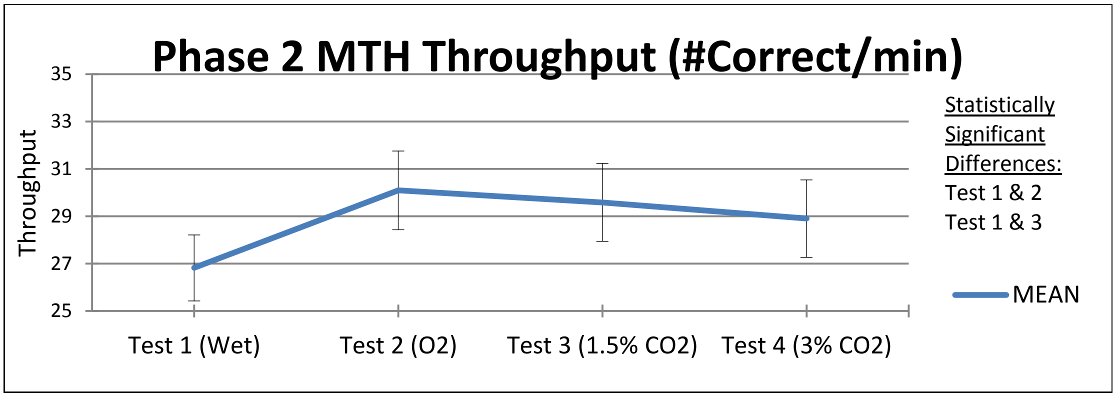


**Figure 8.** Phase 2 MTH throughput (# correct/min). Error bars represent SEM.

CONTINUOUS PERFORMANCE TEST (CPT). Maulchy's test indicated that the assumption of sphericity had not been violated (chi-square = 8.766, p=0.119). Repeated measures ANOVA showed a significant main effect of gas on WM and sustained attention [$F$ (3, 84) = 4.879, p=0.004]. Pairwise analyses revealed that CPT throughput, a measure of WM and sustained attention, was significantly higher (p>0.05) on 3% $CO_2$ than at wet baseline, but not different (p>0.05) from baseline on 1.5% $CO_2$ or on $O_2$. CPT throughput with 3% $CO_2$ was elevated about seven points above wet baseline. However, repeated measures ANOVA across only the postexercise values showed no effect of gas inhaled (Figure 9).

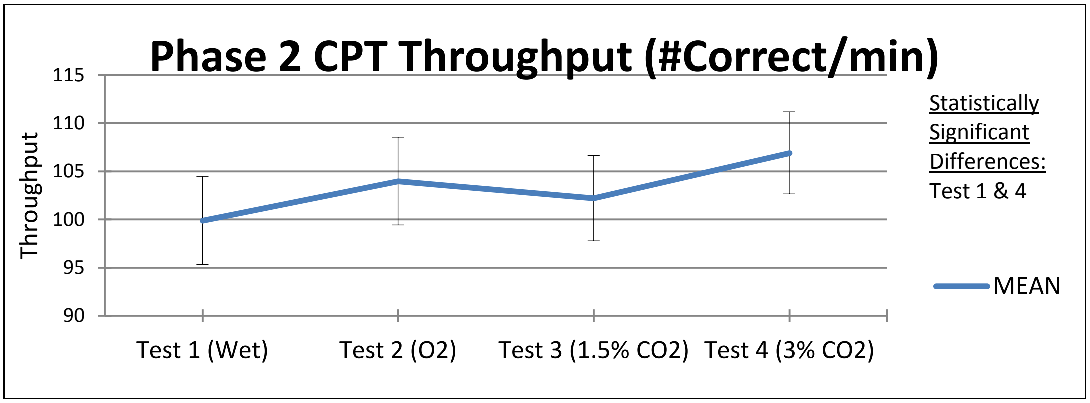

**Figure 9.** Phase 2 CPT throughput (#correct/min). Error bars represent SEM.

Symptoms Related to $CO_2$ Breathing

During Phase 2 the symptom most commonly reported was difficulty in concentrating (Table 11). One or more symptoms were reported by 23 of 35 subjects, again with little apparent relation to elevated $F_{ET}CO_2$ (Table 12). Most symptoms were minor, but one subject aborted his dive, presumably because of the effects of $CO_2$.

That diver had completed his 30-minute period of cycling while breathing 3% $CO_2$. His $F_{ET}CO_2$ had not plateaued during the exercise period and had reached 7.2% when he stopped pedaling, was asked about symptoms, and denied any. Seconds later, while he was moving from the underwater ergometer to the ANAM4 setup, he announced that he was coming up and surfaced rather quickly. After his equipment was removed, he seemed anxious as he tried to formulate his reason for aborting the dive, but clearly he did not really know. Within a few minutes of breathing air, the diver seemed calm, rational, and still confused about what had happened. The only cognitive data available from this diver were the wet baseline, and they were not used in any analyses.

Table 11. Symptoms of $CO_2$ breathing reported during or after Phase 2 dives. Subjects often reported multiple symptoms. Inhaled $CO_2$ when symptom occurred is unknown for some postdive reports.

| **Phase 2: 1.5% and 3% $CO_2$ inhaled in $O_2$** | **n = 34 Overall** | **0% $CO_2$** | **1.5% $CO_2$** | **3% $CO_2$** | **Unknown** |
|---|---|---|---|---|---|
| **Symptom** | **Number of subjects reporting** | | | | |
| Headache | 8 | 1 | 3 | 5 | |
| Shortness of breath | 7 | 1 | 2 | 1 | 3 |
| Poor concentration | 12 | | 1 | 6 | 6 |
| Irritability | 8 | 2 | 2 | 2 | 4 |
| Light-headedness | 2 | 1 | | | 1 |
| Nausea | 1 | | | | 1 |
| Anxiety (abort) | 1 | | | 1 | |

Table 12. Contingency tables: Relation between number of Phase 2 subjects reporting symptoms and number retaining $CO_2$.

| **Phase 2** | $F_{ET}CO_2 \geq 7\%$ | $F_{ET}CO_2 < 7\%$ |
|---|---|---|
| Symptoms | 10 | 13 |
| No symptoms | 2 | 9 |

**PHASE 3**

End Tidal $F_{CO_2}$

With background air during Phase 3, $F_{ET}CO_2$ during exercise increased with inspired $CO_2$, but the apparent increases in resting (postexercise) $F_{ET}CO_2$ with increases in inhaled $CO_2$ were not significant ($p>0.05$; Table 13). No Phase 3 subjects had $F_{ET}CO_2$ values ≥7% at any time.

Table 13. $F_{ET}CO_2$ from Phase 3, mean and standard deviation (% SEV).

| Phase 3 n = 16 | In-water Baseline | Exercise | | | Postexercise rest | | |
|---|---|---|---|---|---|---|---|
| Inspired $CO_2$ | 0% | 0% | 1.5% | 3% | 0% | 1.5% | 3% |
| % SEV | 5.0 (0.4) | 4.9 (0.3) | 5.6 (0.3) | 5.9 (0.4) | 4.9 (0.4) | 5.2 (0.4) | 5.3 (0.4) |

As in Phase 2, with no inspired $CO_2$, $F_{ET}CO_2$ at rest after exercise did not differ ($p>0.4$ by paired t-test) from that before exercise (baseline). $F_{ET}CO_2$ during exercise was significantly higher ($p<0.01$) than that at rest after exercise. During exercise, $F_{ET}CO_2$ was greater ($p<0.01$) with 1.5% $CO_2$ inspired than with no $CO_2$ inspired but did not differ ($p>0.4$) between 1.5% and 3% $CO_2$ inspired, while during postexercise rest, $F_{ET}CO_2$ increased ($p<0.01$) from 0% to 1.5% inspired $CO_2$ and from 1.5% to 3% inspired $CO_2$.

Cognitive Testing

Paired-sample t-tests between dry and wet baselines showed differences only in SRT (Table 14).

Table 14. Phase 3 dry and wet baseline means, SDs, and SEMs.

| Cognitive Subtest | n = 16 | Mean | SD | SEM |
|---|---|---|---|---|
| SS<br>(t = 2.0, p>0.06) | Dry Baseline<br>Wet Baseline | 2.1<br>1.7 | 0.9<br>0.6 | 0.2<br>0.2 |
| SRT (#Correct/min)<br>(t = 3.84, p<0.01) | Dry Baseline<br>Wet Baseline | 228<br>180 | 31<br>42 | 8<br>11 |
| CDS (#Correct/min)<br>(t = 1.72, p>0.1) | Dry Baseline<br>Wet Baseline | 56<br>50 | 16<br>9 | 4<br>2 |
| MTG (#Correct/min)<br>(t = –0.47, p>0.6) | Dry Baseline<br>Wet Baseline | 41<br>42 | 9<br>17 | 2<br>4 |
| M2S (#Correct/min)<br>(t = –0.05, p>0.9) | Dry Baseline<br>Wet Baseline | 39<br>39 | 12<br>12 | 3<br>3 |
| CDD (#Correct/min)<br>(t = –1.08, p>0.2) | Dry Baseline<br>Wet Baseline | 51<br>53 | 12<br>11 | 3<br>3 |
| ST4 (#Correct/min)<br>(t = 1.43, p>0.1) | Dry Baseline<br>Wet Baseline | 93<br>88 | 21<br>18 | 5<br>5 |
| MTH (#Correct/min)<br>(t = 0.64, p>0.5) | Dry Baseline<br>Wet Baseline | 29<br>28 | 7<br>8 | 2<br>2 |
| CPT (#Correct/min)<br>(t = 1.74, p>0.1) | Dry Baseline<br>Wet Baseline | 112<br>104 | 24<br>17 | 6<br>4 |

The unit for SS is response on a scale of 1–7. All other units are for throughput, number correct /minute. Subtests with no significant difference, wet to dry, are shaded.

Repeated measures ANOVA showed no differences within subject (across ANAM tests 1, 2, 3, and 4) in any of the eight cognitive domains other than SS, which was greater with 3% inhaled $CO_2$ than with either 1.5% or 0% inhaled $CO_2$. ANOVA between-subjects effects (air versus $O_2$, with the $O_2$ group taken from Phase 2 with the same order of $CO_2$ presentation) revealed no differences on any of the nine cognitive subtests.

Symptoms Related to $CO_2$ Breathing

Although no Phase 3 subjects had elevated $F_{ET}CO_2$, 12 of 16 subjects reported symptoms that seem to be related to inhaled $CO_2$ (Tables 15 and 16).

Table 15. Symptoms of $CO_2$ breathing reported during or after Phase 3 dives. Subjects often reported multiple symptoms.

| Phase 3: 1.5% and 3% $CO_2$ inhaled in air | n = 16 | 0% $CO_2$ | 1.5% $CO_2$ | 3% $CO_2$ | Unknown |
|---|---|---|---|---|---|
| **Symptom** | **Number reporting** | | | | |
| Headache | 9 | 3 | 4 | 6 | 1 |
| Shortness of breath | 4 | 1 | 3 | 2 | |
| Poor concentration | 4 | | 3 | 2 | |
| Irritability | 2 | | 1 | 1 | |

Table 16. Relation between Phase 3 symptoms and elevated $F_{ET}CO_2$.

| **Phase 3** | $F_{ET}CO_2$ ≥7% | $F_{ET}CO_2$ <7% |
|---|---|---|
| Symptoms | 0 | 12 |
| No symptoms | 0 | 4 |

## SYMPTOMS, ALL PHASES

By Fisher's Exact Test, the proportion of subjects with symptoms did not differ between those breathing $O_2$ or air as background gas (Table 17). The proportion with symptoms was greater ($p<0.01$ by Fisher's Exact Test) for those breathing 3% $CO_2$ (33 of 66 total) than for those breathing 1.5% $CO_2$ (19 of 70; Table 17).

Table 17. Number of subjects reporting symptoms for each gas condition. Some subjects reported symptoms for more than one gas.

| Phase | 3% $CO_2$ | 1.5% $CO_2$ | 0% $CO_2$ | Both $F_{CO_2}$ | Unknown time | No symptoms reported | Number of divers |
|---|---|---|---|---|---|---|---|
| **1a** | N/A | 3 | 0 | N/A | 0 | 17 | 20 |
| **1b** | 4 | N/A | 2 | N/A | 1 | 9 | 16 |
| **2** | 11 | 7 | 4 | 3 | 4 | 11 | 34 |
| **3 (air)** | 8 | 9 | 4 | 7 | 1 | 4 | 16 |

## RESPIRATORY FREQUENCY

Respiratory frequencies were measured from the $CO_2$ traces for Phase 1a, when inspired $CO_2$ was 1.5% SEV, and for Phase 3, when $CO_2$ was inspired in air (Tables 18 and 19). The lowest and highest frequencies seen in Phase 1a were 5 breaths/min and 28 breaths/min, during the last rest period with 100% $O_2$ and during exercise with 1.5%

$CO_2$, respectively. Those in Phase 3 were 3 breaths/min and 29 breaths/min, during rest with air and during exercise with 3% $CO_2$ in air, respectively.

Table 18. Respiratory frequency from Phase 1a, 1.5% $CO_2$ only, mean and standard deviation.

| N = 20 | Exercise | | | Postexercise rest | | |
|---|---|---|---|---|---|---|
| | 0% $CO_2$ | | 1.5% $CO_2$ | 0% $CO_2$ | | 1.5% $O_2$ |
| | 1 | 2 | | 1 | 2 | |
| Frequency (breaths/min) | 13 (4) | 16 (6) | 17 (5) | 10 (4) | 10 (4) | 11 (4) |

Table 19. Respiratory frequency from Phase 3, mean and standard deviation.

| N = 16 | Exercise | | | Post exercise rest | | |
|---|---|---|---|---|---|---|
| | 0% $CO_2$ | 1.5% $CO_2$ | 3% $CO_2$ | 0% $CO_2$ | 1.5% $CO_2$ | 3% $CO_2$ |
| Frequency (breaths/min) | 19 (5) | 18 (5) | 21 (5) | 10 (4) | 13 (5) | 18 (6) |

## PULMONARY FUNCTION, ALL PHASES

No subject reported symptoms of pulmonary oxygen toxicity. All measured changes from baseline are summarized in Table 20, which includes the total number of subjects measured.

Pulmonary function data were pooled for dives with $PO_2$ approximately 1.4 atm (Phases 1 and 2). Immediately after the dives, mean values of flow-volume parameters did not differ from baseline, but on the two days following the dives, FVC was slightly but significantly depressed (Table 20). Of the 68 divers, six had at least one flow-volume parameter below the limits of normal variability, an 8.8% incidence of change in pulmonary function, with binomial 95% confidence interval (C.I.) of 4% to 18%. This incidence is not different from 5% reported in previous four-hour resting or exercise dives for the same $PO_2$.[22,23]

For dives with $PO_2$ approximately 0.3 atm (Phase 3), mean values of FVC and $FEV_1$ were significantly elevated above baseline immediately after surfacing, but mean FVC was slightly but significantly depressed on Day+1 (Table 20). Of the 16 divers, two showed flow-volume parameters depressed below the lower limits of normal variability at any time during testing (incidence 12.5%; C.I., 1% to 39%).

Table 20. Changes in flow-volume parameters after dives. Only statistically significant means ($p<0.05$) and individual values outside normal limits are listed. Note that the means, though significantly depressed, are within normal limits of variability. Diver numbers are arbitrary indications of individuals and are not linked to any other identifiers.

| **$PO_2$ = 1.4 atm** | | | | | | |
|---|---|---|---|---|---|---|
| | **N** | | **FVC** | **$FEV_1$** | **$FEF_{25–75}$** | **$FEF_{max}$** |
| | | Normal variability | 7.7% | 8.4% | 16.8% | 17% |
| Dive day | 68 | Mean | - | - | - | - |
| | | Diver i | –15% | –22% | - | –32% |
| | | Diver ii | - | –12% | - | - |
| Day+1 | 57 | Mean (SEM) | –0.8% (0.4%) p<0.04 | - | - | - |
| | | Diver iii | - | - | –19% | - |
| | | Diver iv | - | –8.9% | - | - |
| | | Diver v | - | –9.6% | - | - |
| | | Diver vi | –7.9% | - | - | - |
| Day+2 | 38 | Mean (SEM) | –1.3% (0.5%) p<0.01 | - | - | - |
| | | Diver vii | - | - | –23% | - |
| | | | | | | |
| **$PO_2$ = 0.3 atm** | | | | | | |
| | **N** | | **FVC** | **$FEV_1$** | **$FEF_{25–75}$** | **$FEF_{max}$** |
| Dive day | 16 | Mean | 2.3 (0.4) p<0.01 | 2.5 (0.9) p<0.01 | - | - |
| Day+1 | 14 | Mean (SEM) | –1.7% (0.6%) p<0.02 | - | - | - |
| | | Diver viii | - | - | –30% | - |
| Day+2 | 8 | Mean | - | - | - | - |
| | | Diver ix | - | - | - | –20% |

## DISCUSSION

### $F_{ET}CO_2$

In subjects at rest or performing mild to moderate exercise, minute ventilation ($V_E$) adjusts to maintain normal $P_aCO_2$ when breathing is unimpeded; $P_aCO_2$ between 35 and 45 Torr, equivalent to $F_{ET}CO_2$ from 4.9 to 6.3% SEV, is considered to be normal.[24] Thus, with unimpeded breathing in subjects with normal lungs who are at rest or are performing light to moderate exercise and are inhaling gas that contains $CO_2$, either $F_{ET}CO_2$ should be unchanged from baseline (because $V_E$ is sufficiently elevated to compensate for the inspired $CO_2$) or $V_E$ will increase when $F_{ET}CO_2$ is elevated in an attempt to compensate. In our subjects exercising underwater, the slightly inadequate increase in $V_E$ when $F_{ET}CO_2$ increased probably occurred because factors in addition to $P_aCO_2$ modulated the drive to breathe. Respiratory frequency, our only indicator of $V_E$, was nowhere near its maximum.

During the light to moderate exercise of this protocol, $F_{ET}CO_2$ increased when subjects inhaled $CO_2$. Some subjects became mildly hypercapnic, and a few accumulated $CO_2$ to the level at which others have reported measurable cognitive effects, $P_{ET}CO_2$ >51 Torr ($F_{ET}CO_2$ >7%).[5] At rest, when ANAM testing was conducted, $F_{ET}CO_2$ increased from that without $CO_2$ during Phase 2 and during Phase 1b (with 3% SEV $CO_2$), but subjects were not hypercapnic. Nevertheless, some small cognitive changes were noted.

Our subjects were exposed to little resistive loading, but they breathed against an inspiratory hydrostatic load while they sat or stood vertically to perform ANAM4 testing. Although the impediment to breathing was small, some subjects retained $CO_2$ and reported symptoms that could be ascribed to hypercapnic effects, and one aborted his dive abruptly and somewhat irrationally. If even a relatively light inspiratory load appears to alter control of ventilation and favor retention of $CO_2$, much more $CO_2$ retention than that we measured is likely with any rebreather UBA. Indeed, considerable $CO_2$ retention has been demonstrated to result from a resistive load similar to that of the MK 16.[25] With increased hypercapnia comes increased concern about its cognitive effects.

### Submersion

Performance underwater could be expected to be degraded relative to that under dry conditions because of more difficulty in seeing the monitor, more difficulty in body positioning, and differences in equipment. Differences, wet to dry, were found in some but not all domains. In the first two phases, fatigue or sleepiness was slightly less in the water than it was in dry status. Reaction time was consistently lower in the submerged than in the dry tests (faster performance), by 28 ms, 69 ms, and 48 ms in Phases 1, 2, and 3, respectively. However, because all in-water testing was compared to wet baseline, the sometimes large submersion effects were factored out of any reported $CO_2$ effects. Thus SRT, which was not different across gas conditions in the water, need not be used to correct other results for within-subject factors.

For Phase 2 only, submersion also decreased CDD, M2S, MTH, and CPT throughputs, by 6, 6, 1, and 12 points, respectively. These changes were not trivial in magnitude: the decrease in CDD from dry to wet baselines was almost as large as that seen with $CO_2$ breathing in Phases 1 and 2, while the 1-point decrease in MTH contrasts with the 2.5- to 2.8-point increases seen during in-water testing. The 12-point decrease in CPT with submersion can be contrasted with the 7-point increases from wet baseline seen with various tests. It can also be compared to the 10- to 15-point decreases reported by others after administration of diphenhydramine (DPH)[26] or during migraine headache.[27] The 6-point decrease in M2S contrasts with a reported decrease of 2.5 to 3 with DPH or migraine, respectively. The causes of these changes are difficult to infer.

### Exercise

Comparisons between the in-water baseline and the first 0% $CO_2$ tests in Phase 1 allow us to infer aftereffects of exercise, while comparisons of wet baseline to other 0% $CO_2$ tests may have confounding effects from intermediary $CO_2$ breathing. However, if ANOVA across all wet conditions indicates a difference, while ANOVA across only postexercise conditions shows no effect of gas inhaled, we can deduce that the effect was one of exercise aftereffects. This argument is strengthened if post hoc pairwise comparisons from the ANOVA with a difference indicate that all the postexercise conditions differ from wet baseline.

In Phase 1 none of the cognitive domains showed an aftereffect of mild exercise. However, in Phase 2 SS was significantly greater (more fatigue) for all in-water gas conditions after exercise than for in-water control and was not different across gases for postexercise conditions (Figure 6). MTH was improved after exercise for 0% and 1.5 % $CO_2$ and not different across gas conditions for postexercise values (Figure 8). The MTH improvement after exercise was more than the decrement caused by submersion.

### Stated Objective 1: Cognitive Effects of 1.5% and 3% SEV-inhaled $CO_2$

We anticipated measurable cognitive effects from 1.5% or 3% SEV-inhaled $CO_2$, because we expected either significant $CO_2$ retention or distraction from high rates of ventilation. In fact, we were concerned that 3% SEV $CO_2$ in inspired gas posed a significant risk to the divers, since fractions of that magnitude have been associated with shallow-water CNS oxygen toxicity in divers using rebreather UBAs.[28–30] However, we saw no overall dose effect of inhaled $CO_2$ on cognitive variables — although there was a dose effect of inhaled $CO_2$ fraction on $F_{ET}CO_2$. On the average, divers were not hypercapnic (Tables 5, 9, and 13), and those few for whom $F_{ET}CO_2$ was >7%, the threshold reported for cognitive effects,[5] showed those elevations during exercise alone, not during ANAM4 testing. Elevated respiratory frequencies (Tables 18 and 19), while noticeable to some divers (Tables 7, 11, and 15), were apparently not overly distracting.

Only one cognitive variable — CDD, a test of LTM — showed a clear decrement with inhaled $CO_2$ in $O_2$. In Phase 1 (Figure 2), with results for the two $CO_2$ fractions pooled, CDD throughput with $CO_2$ was reduced almost eight points from baseline. In Phase 2

(Figure 7), CDD throughput was reduced about 10 points when $CO_2$ was inhaled, with no dose effect and no effect of order of gas presentation.

Three other cognitive variables — ST4, CPT, and MTH — showed changes with inhaled $CO_2$ but gave inconclusive or confusing results.

In Phase 1, ST4, measuring STM, showed a $CO_2$ dose-independent reduction during $CO_2$ inhalation after exercise, but only when the comparison was to 100% $O_2$ after exercise (Figure 3). The 7-point difference between measurements after exercise with $CO_2$ and those after exercise without $CO_2$ was similar in magnitude to a 5-point decrement reported with DPH.[26] However, in Phase 2 ST4 showed no effects of inspired gas. Other researchers[31] also found a change in STM during one test but not during another: when $CO_2$ was elevated to 0.7% during the first 50 minutes of testing in an environmental chamber, STM decreased, but after participants had been in the chamber for two days, increasing $CO_2$ to 1.2% provoked no decrement in STM. While those authors concluded that the initial reduction in STM resulted from a lack of chamber adaptation, this adaptation hypothesis does not apply to our study, in which different subjects were exposed in similar ways in the two phases. It is unclear why Phase 2 and Phase 1 differed.

CPT throughput, a measure of WM, was *higher* (improved) in Phase 2 (Figure 9) with 3% $CO_2$ than during the in-water baseline and was elevated a similar amount (about seven points) during the final $O_2$ period in Phase 1 (Figure 5), while CPT with 1.5% $CO_2$ or 0% $CO_2$ after exercise did not differ from the wet baseline. However, if we lump 1.5% and 3% $CO_2$ in Phase 2 as we did in Phase 1, the average CPT with $CO_2$ was elevated only about four points from the wet baseline, a nonsignificant change similar to that with $CO_2$ in Phase 1. Compared only after exercise in Phase 2, CPT values did not differ from those for inspired gas. Other researchers have shown that both DPH and migraine have significant detrimental effects on CPT: they decrease it 10 and 15 points, respectively.[26,27]

Although MTH throughput in Phase 1 increased above baseline during the final 0% $CO_2$ test (Figure 4), that throughput did not change from baseline with $CO_2$ breathing. However, in Phase 2 MTH improved from wet baseline by 2.8 points with 1.5% $CO_2$ and by 2.1 points (statistically not significant) with 3% $CO_2$, as well as by 3.3 points with 100% $O_2$ (no $CO_2$) after exercise (Figure 8). That improvement is not a learning effect: the order of gas presentation was varied in Phase 2, and the values had plateaued during the dry predive tests. That improvement might be an effect of prior moderate exercise, however, since postexercise values do not differ from one another. Other researchers[31] have similarly reported slight unexplained improvements in mathematical processing (multiplication), an increase in throughput of 1.35/min while subjects were breathing 3% $CO_2$ after two 15-minute periods of treadmill exercise. However, we hesitate to conclude that exercise in conjunction with $CO_2$ increases mathematical processing, because the observed effect size (0.2) was small and the improvements occurred only in Phase 2, with moderate rather than light exercise.

### Stated Objective 2: Off-Effects

In the absence of on-effects, off-effects are difficult to define. The one variable showing a clear $CO_2$ effect, CDD, showed a lingering effect of $CO_2$ after more than 30 min of $O_2$ without $CO_2$ (Figure 7). However, Phase 2 showed no effects from any order of gas presentation.

Some improvements were seen in MTH and CPT from the first to the second periods with 100% $O_2$ in Phase 1 (Figures 4 and 5). This could be interpreted as an off-effect for $CO_2$, but results in Phase 2 were independent of the order of gas presentation. The evidence that these improvements resulted not from a learning effect is that (1) the dry tests before diving showed consistent results on at least the two last tests, and (2) similar improvements in MTH results with 100% $O_2$ were seen in Phase 2, when the order of gas presentation was mixed. It is possible that these improvements were cumulative effects of prior exercise.

### Stated Objective 3: Effects of $PO_2$ on Hypercapnic Effects

Although in-water baseline $F_{ET}CO_2$ was identical with air or $O_2$, $F_{ET}CO_2$ values with inhaled $CO_2$ were lower with background air than with background $O_2$ (Tables 9 and 13). In healthy subjects breathing of hyperoxic gas is known to stimulate $V_E$ if normocapnia can be maintained,[9] but hyperoxia also blunts the ventilatory response to $CO_2$.[33] The increased effectiveness of $V_E$ with air rather than with $O_2$ suggests that the peripheral chemoreceptors, the responses of which are blunted by high $PO_2$, help to drive ventilation in these subjects.

In Phase 3 none of the cognitive variables except SS showed significant differences with inhaled $CO_2$. Because $F_{ET}CO_2$ in this phase was closer to normal in all subjects (max 6.5% SEV), the lack of change in cognitive variables lends credence to the idea that some of the effects observed in Phases 1 and 2 are the results of retained $CO_2$. But this lack of change adds doubt to the idea that any of the responses was an aftereffect of exercise.

Since all cognitive measurements made while subjects breathed $CO_2$ in air were made during normocapnia, we cannot comment on the effects of $PO_2$ during hypercapnia.

### Overall

When cognitive function on inspired $CO_2$ after exercise was compared with that on $O_2$ before exercise (wet baseline), LTM was impaired regardless of the intensity of exercise (mild or moderate, corresponding to Phases 1 and 2, respectively). Fatigue, mathematical processing, WM, and sustained attention were all enhanced with moderate, but not mild, exercise. When cognitive function on inspired $CO_2$ after exercise was compared with that on $O_2$ after exercise, STM was impaired only after mild (Phase 1) but not after moderate exercise (Phase 2). However, since we found no difference in STM between $O_2$ after exercise and the wet baseline, we cannot attribute the difference to effects of exercise. When we compared $O_2$ before exercise (test 1, wet baseline) with $O_2$ after exercise (test 2), only fatigue and mathematical processing were enhanced, both with moderate (Phase 2) but not mild (Phase 1) exercise.

After one hour of breathing 1.5% or 3% SEV $CO_2$ with 30 minutes of exercise, mathematical processing, WM, and sustained attention while breathing 100% $O_2$ improved (test 4 in Phase 1) from those levels during a similar period of breathing 100% $O_2$ before the $CO_2$ (test 2 in Phase 1). We cannot determine whether the $CO_2$ was implicated. Although elevated arterial $P_{CO_2}$ increases brain blood flow, we have no evidence that this is implicated and cannot clearly explain why any cognitive function would increase while breathing $CO_2$ in $O_2$.

**Symptoms of Hypercapnia**

Although objective effects were few, inhaled 1.5% or 3% SEV $CO_2$ was not harmless. A total of 23 of 86 subjects reported symptoms associated with breathing 3% $CO_2$, and 19 subjects reported symptoms associated with breathing 1.5% $CO_2$ across all three phases of the study. This number was significantly more with 3% than with 1.5% inspired $CO_2$. Ten subjects from Phases 2 and 3 reported symptoms with both inhaled $CO_2$ fractions. Symptoms included headache, a subjective sense of difficulty concentrating, irritability, and, in one case, anxiety that preceded the sudden termination of a dive. The fraction of the subjects with symptoms did not differ with levels of $P_{O_2}$. The presence of symptoms may be a more sensitive indicator of $CO_2$ retention than are the cognitive measures, and some symptoms may be caused by the increased breathing necessary to maintain normal $F_{ET}CO_2$.

**Pulmonary Function**

The small fractions of $CO_2$ in the inspired gas do not dilute the background breathing gas to any appreciable extent, and thus effects of $CO_2$ on pulmonary function were expected only if pulmonary arterial vasoconstriction became prominent. At $P_{O_2}$ = 1.4 atm, the incidence of changed flow-volume parameters after 3.5-hour dives with mild to moderate exercise was not different from that previously reported for four-hour resting or exercise dives at the same $P_{O_2}$.[22,23] Similarly, at $P_{O_2}$ = 0.3 atm the incidence of changes in flow-volume parameters did not differ from that for four-hour resting dives with $P_{O_2}$ = 1.4 atm. The increase measured in pulmonary function parameters in Phase 3, the 3.5-hour air dives, is in concordance with an increase measured but not noted in previous four-hour air dives.[22,23]

The absence of reported symptoms of pulmonary oxygen toxicity after these dives differs from previous results, in which 17% to 23% of divers reported mild symptoms after single four-hour dives with $P_{O_2}$ = 1.3 atm.[22,23] This absence of symptoms could be real, but it could also represent reporting bias in a study where so many questions were asked about subjective effects of $CO_2$ that “ordinary” respiratory symptoms may have been ignored or denied by the subjects. In one series of air dives with 14 subjects, no symptoms were reported,[22] while the incidence of symptoms in another series was not different with air or $O_2$ as the breathing gas.[19]

### Methodological Weaknesses

While taking the ANAM4, divers were not exercising. That is, cognitive performance was not assessed during exercise. In operational settings, however, exercise and cognitive function are not independent: if a diver's concentration and sustained attention are jeopardized while he or she is swimming during a mission, it may have tremendous implications for the success of the mission and the safety of the diver and the crew. Since most elevations in $F_{ET}CO_2$ occurred only during exercise and a elevation in $F_{ET}CO_2$ may betoken variations in cognitive performance, future studies should investigate these two occurrences in tandem.

A confounder was continued time underwater, with the attendant physiological changes and problems of thermal control. Phase 2 attempted to balance these effects by presenting each gas condition at all possible times during the experiment.

## CONCLUSIONS

### Stated Objectives

Dose Response, $CO_2$ Response, Off-response, $P_{O_2}$ Effects

No dose-related effects of inspired $CO_2$ on cognitive function were measured; results for none of the nine cognitive domains differed between 1.5% and 3% SEV inspired $CO_2$. However, more symptoms were reported with 3% than with 1.5% SEV inspired $CO_2$.

As measured by CDD, both doses of $CO_2$ in $O_2$ consistently depressed LTM, while $CO_2$ in air did not affect any of the investigated cognitive domains except sleepiness/fatigue.

Evidence for lingering effects of $CO_2$ is mixed. In Phase 1 CDD did not return to baseline, but MTH and CPT increased relative to baseline when $CO_2$ was removed. In Phase 2, the order of gas presentation did not affect the results.

Maintenance of normal arterial $P_{CO_2}$ may have protected cognitive function. Although the average increase in $F_{ET}CO_2$ with either inhaled gas concentration was higher during exercise than at rest and higher with 3% than with 1.5%, it was a modest increase at most, with the breathing gear used here. Average $F_{ET}CO_2$ during ANAM4 testing was not different from baseline with inspired $CO_2$ in Phase 1a (Table 5) or Phase 3 (Table 13).

We cannot address the major question of the study, the effects of hypercapnia on cognitive function. However, we have investigated the effects of inhaled $CO_2$ when divers are not hypercapnic. No conclusions from this work should be applied to situations in which inspiration of 1.5% or 3% $CO_2$ is expected to provoke $CO_2$ retention.

This study was a wide-stroke approach to cognition and diving. Since this study has found effects on higher executive functions of LTM, WM, sustained attention, and math processing in conjunction with inspired $CO_2$, mild-moderate exercise, and submersion, future studies can target these variables with a more focused approach.

## RECOMMENDATIONS

A relaxing of $CO_2$ limits for rebreather diving has been discussed.[1] However, even with the MK 20's low breathing resistance and the resultant small increases in $F_{ET}CO_2$ with increased inspired $CO_2$, we measured a decrease in LTM associated with inspired $CO_2$. Some subjects also subjectively reported decreases in their abilities to concentrate and increases in irritability, and one subject abruptly aborted his dive.

We recommend that no changes be made to the limits for inspired $CO_2$ from absorbent canisters until further investigation is made. Two specific measurements are needed before recommendations can be made:

> (1) The combined effects of exercise and inspired $CO_2$ on the higher executive functions of WM, sustained attention, mathematical processing, and LTM should be assessed.
>
> (2) Cognitive measurements should be made with inspired $CO_2$ when breathing resistance is elevated.

## REFERENCES


1. M. E. Knafelc, *Physiological Basis for $CO_2$ Limits within Semiclosed- and Closed-Circuit Underwater Breathing Apparatus*, NEDU TR 4-00, Navy Experimental Diving Unit, Panama City, FL, Aug 2000.

2. R. A. Henning, S. L. Sauter, E. H. Lamphier, and W. G. Reddan, “Behavioral Effects of Increased $CO_2$ Load in Divers,” *Undersea Biomed. Res.*, Vol. 17, No. 2 (1990), pp. 109–120.

3. D. M. Fothergill, D. Hedges, and J. B. Morrison, “Effects of $CO_2$ and $N_2$ Partial Pressure on Cognitive and Psychomotor Performance,” *Undersea Biomed. R.*, Vol. 18, No. 1 (1991), pp. 1–18.

4. Commander, Naval Sea Systems Command, *U.S. Navy Diving Manual, Revision 6* (Arlington, VA: NAVSEA, 2008), Chapter 3.

5. J. A. Sayers, R. E. A. Smith, R. L. Holland, and W. R. Keatinge, “Effects of Carbon Dioxide on Mental Performance,” *J. Appl. Physiol.*, Vol. 63, No. 1 (1987), pp 25–30.

6. S. J. Menn, R. D. Sinclair, and B. E. Welch, “Effect of Inspired $P_{CO_2}$ up to 30 mm Hg on Response of Normal Man to Exercise,” *J. Appl. Physiol*., Vol. 28, No. 5 (1970), pp. 663–671.

7. D. Kerem, Y. Melamed, and A. Moran, “Alveolar $P_{CO_2}$ During Rest and Exercise in Divers and Non-divers Breathing $O_2$ at 1 ATA,” *Undersea Biomed. Res*., Vol. 7, No. 1 (1980), pp. 17–26.

8. U.S. Naval Sea Systems Command, *Task Assignment 09-01: Cognitive Effects of Hypercapnia*, Ser 00CM/0012, 19 Feb 2009.

9. H. F. Becker, O. Pollo, S. G. McNamara, M. Berthon-Jones, and C. E. Sullivan, “Effect of Different Levels of Hyperoxia on Breathing in Healthy Subjects,” *J. Appl. Physiol.*, Vol. 81, No. 4 (1996), pp. 1683–1690.

10. M. Lowe and D. Reeves, *ANAM Diving Medicine Subset (DMS) User’s Manual*, NEDU TR 03-01, Navy Experimental Diving Unit, May 2003.

11. M. A. Lowe and D. L. Reeves, “Accelerated Decompression from Nitrox Saturation Diving: Neuropsychological Findings,” *Undersea and Hyperbaric Medicine*, Vol. 27 (Supplemental, 2000), p. 15.

12. M. A. Lowe and D. L. Reeves, *Deep Dive 1998: Neuropsychological Findings*, NEDU TR 13-01, Navy Experimental Diving Unit, Nov 2001.

13. M. A. Lowe, D. L. Reeves, and R. Kane, “At Depth Computerized Assessment of Neurocognitive Changes in Divers,” *Archives of Clinical Neuropsychology*, Vol. 15, No. 8 (November 2000), p. 699.

14. M. A. Lowe, D. L. Reeves, and E. Long, *Warm Water Diving 1999: Neuropsychological Findings*, NEDU TR 02-11, Navy Experimental Diving Unit, Sep 2002.

15. M. A. Lowe and D. L. Reeves, *Automated Neuropsychological Assessment Metrics: Norms for U.S. Navy Divers*, NEDU TR 02-05, Navy Experimental Diving Unit, May 2002 (Unclassified).

16. D. L. Reeves, K. P. Winter, J. Bleiberg, and R. L. Kane, “ANAM Genogram: Historical Perspectives, Description, and Current Endeavors,” *Archives of Clinical Neuropsychology*, Vol. 22S (2007), pp. S15–S37.

17. A. Collie, P. Maruff, D. G. Darby, and M. McStephen, “The Effects of Practice on the Cognitive Test Performance of Neurologically Normal Individuals Assessed at Brief Test-Retest Intervals,” *Journal of The International Neuropsychological Society*, Vol. 9 (2003), pp. 419–428.

18. D. R. Thorne, “Throughput: A Simple Performance Index with Desirable Characteristics,” *Behavior Research Methods*, Vol. 38, No. 4 (2006), pp. 569–573.

19. B. E. Shykoff, *Pulmonary Effects of Submerged Breathing of Air or Oxygen*, NEDU TR 02-14, Navy Experimental Diving Unit, Nov 2002.

20. B. E. Shykoff, *Underwater Cycle Ergometry: Power Requirements with and without Diver Thermal Dress*, NEDU TR 09-01, Navy Experimental Diving Unit, Jan 2009.

21. A. Selkirk, *Equipment Setup Instructions for the Computerized Underwater Cognitive Assessment, Version 2009*, NEDU TM 09-10, Navy Experimental Diving Unit, Oct 2009.

22. B. E. Shykoff, "Pulmonary Effects of Submerged Exercise While Breathing 140 KPa Oxygen," *Undersea and Hyperbaric Medicine*, Vol. 35, No. 6 (Nov/Dec 2008), pp. 417–426.

23. B. E. Shykoff, "Pulmonary Effects of Submerged Oxygen Breathing in Resting Divers: Repeated Exposures to 140 KPa," *Undersea and Hyperbaric Medicine*, Vol. 35, No. 2 (2008), pp. 131–143.

24. M. G. Levitsky, *Pulmonary Physiology* (New York: McGraw-Hill, 1982), p. 159.

25. B. Shykoff, D. Warkander, and D. Winters, "Effects of Carbon Dioxide and UBA-like Breathing Resistance on Exercise Endurance," NEDU TR 10-03, Navy Experimental Diving Unit, Apr 2010.

26. J. A. Wilken, C. L. Sullivan, A. Lewandowski, and R. L. Kane, "The Use of ANAM to Assess the Side-effect Profiles and Efficacy of Medication," *Archives of Clinical Neuropsychology*, Vol. 22S (2007), pp. S127–S133.

27. T. Roebuck-Spencer, W. Sun, A. N. Cernich, K. Farmer, and J. Bleiberg, "Assessing Change with the Automated Neuropsychological Assessment Metrics (ANAM): Issues and Challenges," *Archives of Clinical Neuropsychology*, Vol. 22, Suppl. 1 (Feb 2007), pp. 79–87.

28. R. Arieli, T. Shochat, and Y. Adir, "CNS Toxicity in Closed-circuit Oxygen Diving: Symptoms Reported from 2527 Dives," *Aviation, Space, and Environmental Medicine*, Vol. 77, No. 5 (May 2006), pp. 526–532.

29. R. Arieli, G. Rashkovan, Y. Moskovitz, and O. Ertracht, "$P_{CO_2}$ Threshold for CNS Oxygen Toxicity in Rats in the Low Range of Hyperbaric $P_{O_2}$," *J. Appl. Physiol.*, Vol. 91 (2001), pp. 1582–1687.

30. R. Arieli, Y. Arieli, Y. Daskalovic, M. Eynan, and A. Abramovich, "CNS Oxygen Toxicity in Closed-circuit Diving: Signs and Symptoms before Loss of Consciousness," *Aviat., Space, and Environ. Med.*, Vol. 77, No. 11 (2006), pp. 1153–1157.

31. D. Manzey, B. Lorenz, and G. Finell, "Effects of $CO_2$ on Cognitive, Psychomotor, and Time-sharing during 26 Days of Confinement," *ASGSB Bull*., Vol. 9 (Oct 1995), p. 59.

32. M. Vercruyssen, E. Kamon, and P. Hancock, "Effects of Carbon Dioxide Inhalation on Psychomotor and Mental Performance during Exercise and Recovery," *Int. J. of Occupational Safety and Ergonomics (JOSE)*, Vol. 13, No. 1 (2007), pp. 15–27.

33. A. Dahan, J. DeGoede, A. Berkenbosch, and I. C. W. Olievier, "The Influence of Oxygen on the Ventilatory Response to Carbon Dioxide in Man," *J. Physiol.*, Vol. 428 (1990), pp. 485–499.